Pharmacokinetics of Acetylsalicylic Acid and Salicylic Acid After Intravenous Administration in Man

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The pharmacokinetics of acetylsalicylic acid (ASA, 650 mg.) and salicylic acid (SA, 500 mg.) were studied following intravenous administration in males. The resultant plasma concentration-time curves were described by bi-exponential equations. The half-life of the first exponent was 2–5 min. for both compounds while that of the second exponent was 13–19 min. and 3.5–4.5 hr. for ASA and SA, respectively. Over the dose range 0.3–1.2 g. ASA, the area under the ASA plasma concentration-time curve was proportional to the dose administered. Also SA was shown to be the exclusive metabolite of ASA. Analysis of the present results, over the dose range and duration of study, showed that the data could best be fitted by conceiving the body to act as a two-compartmental open system with respect to these drugs. The significance of these findings on measurement of the rate constants of metabolism, volume of distribution, and other pharmacokinetic parameters are discussed.

It is an interesting fact that, although acetylsalicylic acid (ASA) has been consumed in tremendous quantities since its introduction in 1899, only scant information is available pertaining to the pharmacokinetics of this drug in man. This is all the more surprising as the salicylates have been the subject of two monographs (1, 2) and a symposium (3). Essentially, the problem has been an analytical one in that previous assays either did not differentiate between ASA and its metabolite, salicylic acid (SA), or estimated ASA as the increase in SA upon hydrolysis of the sample (4, 5), which is at best an insensitive method. More recently attempts have been made to increase the specificity of the assay using paper chromatography or selective extraction (6, 7).

Although ASA is known to be rapidly metabolized to SA *in vivo*, only the mean data of Leonards (8) and Lange and Bell (6) allow any estimate for the apparent elimination half-life, and values of 17 min. and 30 min. are obtained, respectively. However, in both studies, the drug was given orally so that estimates could be influenced by continued absorption of ASA during the decline of drug blood levels. The total urinary recovery of ASA as SA or its metabolites (9) suggests that SA is the only metabolite of ASA while 1.5% has been reported to be excreted unchanged in the urine of man (10). Much more information is available relating to SA owing to its high plasma levels, relatively long half-life, and ease of analysis (1–3). Nevertheless, there are little data on the kinetics of distribution of this drug in man.

In the present paper both ASA and SA have been given intravenously to man as their *N*-methylglucamine salt. The kinetics of distribution, metabolism, and excretion of each drug was then determined. Over the duration of this study it was found that the results can only be adequately explained by proposing as a minimum a two-compartmental open-system model for each drug. A preliminary communication of these results have been reported elsewhere (11).

EXPERIMENTAL

The dose of either ASA or SA was administered as its *N*-methylglucamine salt. Equivalent amount of acid and base were mixed together and dissolved in water for injection so that each dose was contained in 10-ml. final product. The solutions were passed through a bacterial filter into a sterile, multidose container and used immediately after preparation. The solutions were assayed for SA, and ASA, by UV, spectrophotofluorometric, and gas-liquid chromatographic procedures to verify contents.

Five male subjects were used in the study and each received a rapid intravenous injection (over 5–10 sec.) of 650 mg. ASA while on another occasion four of these subjects received 500 mg. SA. Blood samples (5 ml.) were usually taken at 0, 1, 2, 4, 6, 8, 10, 12, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 105, 120, 180, 240, 300, 360, and 480 min. following ASA administration, while slightly less frequent samples were taken in the SA study. In addition one subject was given 325 mg. ASA i.v. and in another study received a zero-order i.v. infusion of 1.24 g. ASA over a 50-min. period using a Harvard infusion pump. In the ASA study the collected blood samples were immediately placed into cooled tubes containing heparin and potassium fluoride (which inhibits further hydrolysis of ASA) and centrifuged. The plasma was then analyzed specifically for ASA using gas-liquid chromatography

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while SA was determined spectrophotofluorometrically (12).

RESULTS AND DISCUSSION

In all subjects the ASA plasma-concentration-time curve following 650 mg. ASA i.v. was described by a bi-exponential equation of the form $C_p = Ae^{-\alpha t} + Be^{-\beta t}$ (*e.g.*, Fig. 1).[1] Both the initial distribution phase and the so-called elimination phase are extremely short with average half-lives of 2.8 and 15 min., respectively (Table I). Consequently, no ASA is present in the body 1 hr. following the intravenous dose. The rapid *in vivo* hydrolysis of ASA to SA causes the SA plasma level to rise sharply (40–50 mcg./ml.) and owing to its metabolic stability, the disposition half-life[2] was 3.0–4.5 hr. in this study, SA remains in the body for a long time after all the ASA has been eliminated.

The bi-exponential plasma curve can be interpreted in terms of a two-compartmental open system depicted as in Fig. 2.

In this model k_{12}, k_{21}, are the first-order rate constants for distribution of the drug between the central and so-called peripheral or tissue compartment while k_{13} is the elimination (metabolism and excretion) rate constant. V_p,C_p are the volume of the central compartment and the plasma concentration of drug, respectively, while V_T,C_T are the corresponding values for the tissue compartment (expressed relative to the central compartment). It follows from this model that dose administered = $V_pC_p^0$, where C_p^0 is the plasma concentration at zero time and can be seen to be numerically equal to the sum of the coefficients A and B in the bi-exponential equation describing the plasma concentration-time curve. The values of k_{12}, k_{21}, and k_{13} are calculated from A, B, α, and β (13). Also the model shows ASA distributing between two compartments while metabolism and excretion occur only in the central compartment, which includes the liver, kidneys, as well as blood. Needless to say, the model is an over-simplification of the body, but it is the minimum model required to fit the observed ASA data. Having assumed such a model, the various parameters were determined for each subject (Table II). As expected the distribution rate constants k_{12}, k_{21} are extemely fast with mean half-lives of 8.6 min. and 5.8 min. (*i.e.*, $0.693/k_{12}$, $0.693/k_{21}$). More noteworthy is the value of the elimination rate constant k_{13}, which is of the same magnitude as k_{12} and k_{21}, and is approxmately twice that of the disposition rate constant β (Table I). In fact, the only reason that the latter phase has a half-life as long as 15 min. ($0.693/\beta$) instead of 6.7 min. ($0.693/k_{13}$) is due to distribution of ASA into the peripheral compartment so that effectively less is available for immediate metabolism.

In further experiments subject A received 325 mg., 650 mg., and 1.24 g. ASA i.v. on separate occasions and the ASA areas (area/g. dose) were 1460, 1460, and 1390, respectively. Thus, proportionality between area and dose exists over the range studied and could only occur if the rate constants do not change with dose. Since the disposition half-life of SA is dose dependent, increasing with higher doses, proportionality between SA area and dose is not expected.

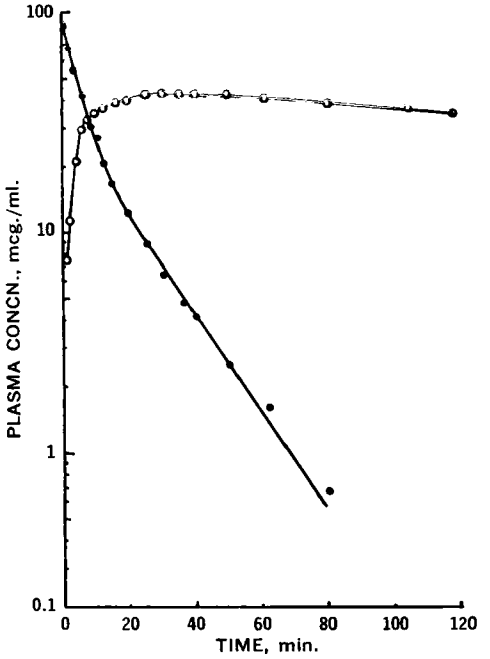

Fig. 1—Graph showing plasma concentrations of ASA and SA following i.v. administration of 650 mg. ASA. Subject D. Key: ●, ASA; ○, SA.

TABLE I—BI-EXPONENTIAL EQUATIONS DESCRIBING THE LOSS OF DRUG FROM PLASMA, THE HALF-LIVES OF THE DISTRIBUTION AND DISPOSITION PHASES OF ASA, TOGETHER WITH THE AREA UNDER THE PLASMA CONCENTRATION-TIME CURVE OF ASA FOLLOWING AN I.V. DOSE OF ASA IN MAN

Subject	Dose, mg.	$C_p = Ae^{-\alpha t} + Be^{-\beta t}$, t in min.	Half-Lives[a] min. 0.693/α	0.693/β	Area ($\int_0^{\infty} C_p dt$), mcg. min. ml.$^{-1}$
A	325	$42e^{-0.26t} + 15.5e^{-0.048t}$	2.7	14.5	480
	650	$90e^{-0.24t} + 29e^{-0.050t}$	2.8	15.0	960
B	650	$56e^{-0.31t} + 37e^{-0.050t}$	2.2	13.7	910
C	650	$60e^{-0.29t} + 40e^{-0.048t}$	2.4	14.5	1000
D	650	$67e^{-0.23t} + 33e^{-0.050t}$	3.0	14.0	960
E	650	$64e^{-0.19t} + 22e^{-0.037t}$	3.7	19.0	950
Mean			2.7	14.9	—

[a] Mean half-life values in this and subsequent tables are harmonic means.

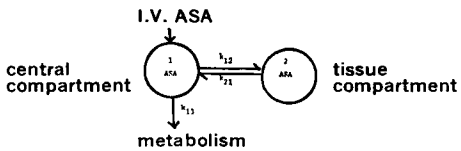

Fig. 2—Schematic diagram showing the distribution and metabolism of ASA following i.v. administration.

[1] As discussed by Riegelman *et al.* (13), the terms α and β are the fast and slow disposition rate constants, respectively, while the term disposition half-life is the value $0.693/\beta$ referred to by others as the elimination or biological half-life.

[2] The authors observed, among others (14,15), that the disposition half-life of SA varies with dose. The half-life values reported here are those obtained following either 650 mg. ASA or 500 mg. SA.

TABLE II—ESTIMATES FOR THE RATE CONSTANTS AND HALF-LIVES OF DISTRIBUTION AND ELIMINATION FOR ASA IN MAN, ASSUMING A TWO-COMPARTMENTAL OPEN SYSTEM

Subject	Dose of ASA, mg.	Rate Constants, min.$^{-1}$ k_{12}^{ASA}	k_{21}^{ASA}	k_{13}^{ASA}	Half-Lives, min 0.693/k_{12}^{ASA}	0.693/k_{21}^{ASA}	0.693/k_{13}^{ASA}
A	325	0.083	0.104	0.117	8.4	6.7	5.7
	650	0.073	0.097	0.123	9.5	7.2	5.6
B	650	0.105	0.157	0.110	6.7	4.2	6.2
C	650	0.095	0.152	0.091	7.3	4.6	7.6
D	650	0.067	0.109	0.105	10.4	6.4	6.6
E	650	0.058	0.074	0.092	12.1	9.4	7.0
Mean		0.081	0.119	0.103	8.6	5.8	6.7

TABLE III—BI-EXPONENTIAL EQUATIONS DESCRIBING THE LOSS OF DRUG FROM PLASMA AND THE HALF-LIVES OF DISTRIBUTION AND DISPOSITION PHASES OF SA FOLLOWING AN I.V. DOSE OF SA IN MAN

Subject	Dose, mg.	$C_p = Ae^{-\alpha t} + Be^{-\beta t}$ t in min.	Half-Lives, min. 0.693/α	0.693/β
A	508	$38e^{-0.17t} + 58e^{-0.0027t}$	4.1	256
B	484	$32e^{-0.22t} + 50e^{-0.0026t}$	3.2	266
C	500	$44e^{-0.23t} + 60e^{-0.0028t}$	3.0	210
D	508	$40e^{-0.14t} + 49e^{-0.0032t}$	5.0	216
Mean			3.8	238

Before attempting to interpret the SA plasma levels resulting from the ASA dose, SA was given intravenously. A dose level of 500 mg. was chosen as this would result from complete hydrolysis of 650 mg. ASA. The resulting plasma levels, like those of the ASA study, showed a bi-exponential curve in the four subjects examined. However, whereas the distribution half-life (3.8 min.) is similar to ASA, the disposition half-life[3] is much longer (Table III). As with ASA, the results were interpreted in terms of a two-compartmental model, and the corresponding rate constants k_{12}, k_{21}, and k_{13} were calculated (Table IV). These analyses confirm that the major difference between ASA and SA is one of metabolic stability since the corresponding k_{12}, k_{21} values of SA and ASA for each subject are similar whereas the k_{13}^{ASA} is 15 times k_{13}^{SA}.

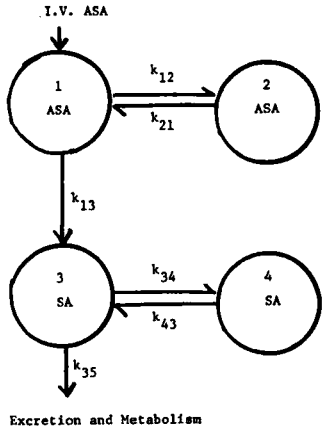

Fig. 3—Schematic diagram for the distribution and elimination of ASA and SA following an i.v. dose of ASA (650 mg.).

this model k_{13} is shown to be the only route of elimination of ASA and to be equal to the rate constant for conversion of ASA to SA. Obviously urinary excretion of unchanged drug and other metabolites are also possibilities. However, the contribution of excretion is small as only 1.5% of the dose has been reported to be excreted unchanged and may be ignored (10). The second possibility was discounted

TABLE IV—ESTIMATES FOR THE RATE CONSTANTS AND HALF-LIVES OF DISTRIBUTION AND ELIMINATION FOR SA IN MAN ASSUMING A TWO-COMPARTMENTAL OPEN SYSTEM

Subject	Dose SA, mg.	Rate Constants, min.$^{-1}$ k_{12}^{SA}	k_{21}^{SA}	k_{13}^{SA}	Half-Lives, min. 0.693/k_{12}^{SA}	0.693/k_{21}^{SA}	0.693/k_{13}^{SA}
A	508	0.070	0.102	0.0042	9.9	6.8	165
B	484	0.085	0.133	0.0040	8.1	5.2	173
C	500	0.097	0.131	0.0056	7.2	5.3	124
D	508	0.061	0.078	0.0058	11.3	8.9	120
Mean		0.078	0.110	0.0049	9.1	6.2	142

From the foregoing results and discussion, the scheme in Fig. 3 is proposed to describe the pharmacokinetics of ASA and SA following an intravenous injection of ASA to man. In this diagram, $k_{34} = k_{12}^{SA}$, $k_{43} = k_{21}^{SA}$ and $k_{35} = k_{13}^{SA}$ as in Table IV. In when calculations showed that the SA area[4] following 650 mg. ASA was equal to that obtained from 500 mg. SA administration (Table V). Since 650 mg. ASA is equivalent to 500 mg. SA the above result can only occur when SA is the sole metabolite of ASA,

[3] After the distribution phase the SA plasma levels for the 8-hr. period over which most studies were conducted could be fitted by a straight line when plotted on semilogarithmic paper. Literature evidence (16) and the authors' observations, however, show that SA plasma levels exhibit a curvature if followed long enough. Hence the β^{SA} and k_{13}^{SA} values calculated are only apparent constants but are adequate for describing the levels observed during the course of these experiments.

[4] In calculating the total area under the SA concentration time curve, it was assumed that the observed apparent disposition half-life (0.693/β) would not change with time so that the extra area subsequent to the 8-hr. study is determined by $C_{8A} \cdot 1/\beta$ where C_{8A} is the last plasma data point. It was reasoned that even if curvature of the plasma SA took place it would be similar for both i.v. SA and SA produced from ASA, so that the error introduced in comparing the relative areas would not be appreciable.

and therefore, k_{13} has the meaning depicted in Fig. 3.

The pharmacokinetics of these salicylates were studied further using a PACE TR 48 analog computer (the differential equations and analog diagram of which are given in the appendix). As expected the computer curves fitted the observed i.v. ASA, and SA data (Fig. 4). Additional evidence in favor of the proposed model (Fig. 3) was obtained when, using the rate constants calculated for each subject (except E which received no i.v. SA), there was an excellent fit between observed and predicted SA levels resulting from i.v. ASA, *e.g.*, Fig.

TABLE V—COMPARISON OF THE AREA UNDER THE SALICYLIC ACID PLASMA CONCENTRATION-TIME CURVE FOLLOWING 650 MG. ASA AND 500[a] MG. SA I.V.

Subject	SA Area After 650 mg. i.v. ASA, mcq./min. ml.$^{-1}$	SA Area After 500 mg. i.v. SA, mcq./min. ml.$^{-1}$	Ratio of Areas × 100
A	22,000	20,600	107
B	20,300	20,100	101
C	21,700	21,400	101
D	14,100	15,000	94
Mean			101

[a] Areas corrected to a 500-mg. SA dose.

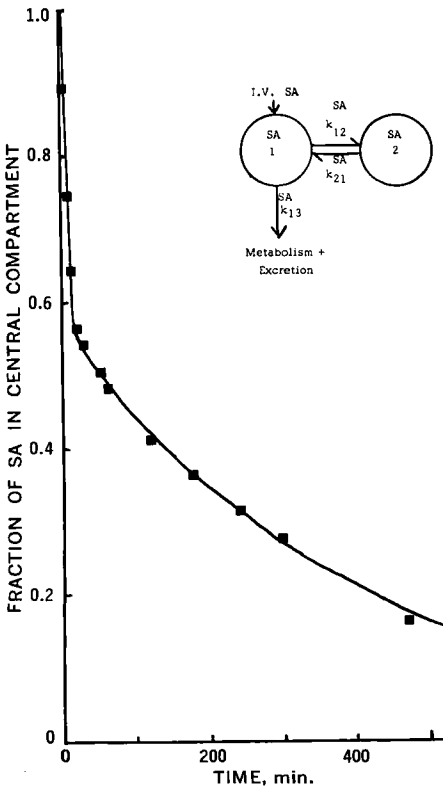

Fig. 4—Graph showing the observed and predicted values for the fraction of SA in the central compartment following 484 mg. i.v. SA administration. Subject B. Key: ■, experimental SA; ———, predicted analog computer curve.

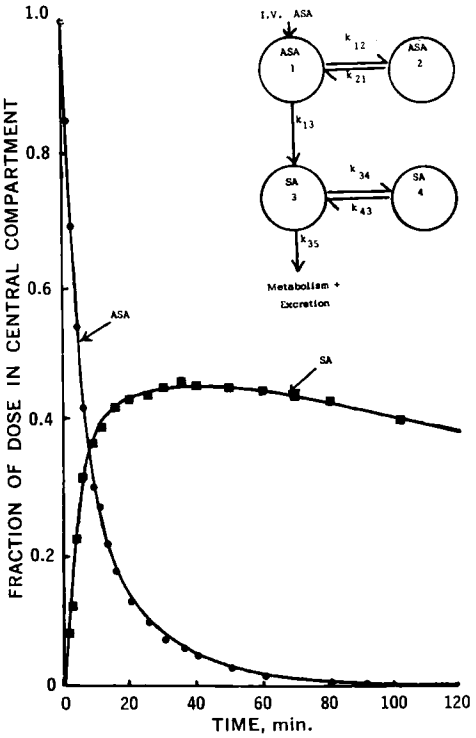

Fig. 5—Graph showing the observed and predicted values for the fraction of the dose as ASA and SA in the central compartment following i.v. administration of 650 mg. ASA. Subject D. Key: ●, experimental ASA; ■, experimental SA; ———, predicted analog computer curves.

5. It should be noted that in Fig. 4 and in subsequent analog computer curves the concentration terms have been replaced by the fraction of each drug present in the central compartment, otherwise overloading of the computer can occur. For the intravenous doses the fraction term is simply obtained by dividing the plasma concentration by the corresponding value of C_p^0. The SA data obtained from i.v. ASA were normalized by making the maximum SA plasma concentration equal to the peak fraction of the dose as SA in the central compartment which had been obtained from the analog computer curves.

It is apparent that an alternative model, in which ASA hydrolysis takes place exclusively in the peripheral compartment or partially in both, may also account for the observed data. The former was eliminated since it is known that ASA is hydrolyzed in whole blood which is an essential part of the central compartment. The latter model was further examined using the analog computer and like the model depicted in Fig. 3 it also fitted the ASA plasma data (*e.g.*, Fig. 6). However, unlike the originally proposed model, the one assuming partial peripheral metabolism resulted in a lower rate of appearance of plasma SA than the experimental data. Any attempts at varying the SA rate constants, while maintaining the same ASA constants determined for the model involving peripheral metabolism of ASA (model B, Fig. 6), in order to increase the rate of appearance of plasma SA but still fitting the i.v. SA data, proved unsuccessful in all subjects. It was,

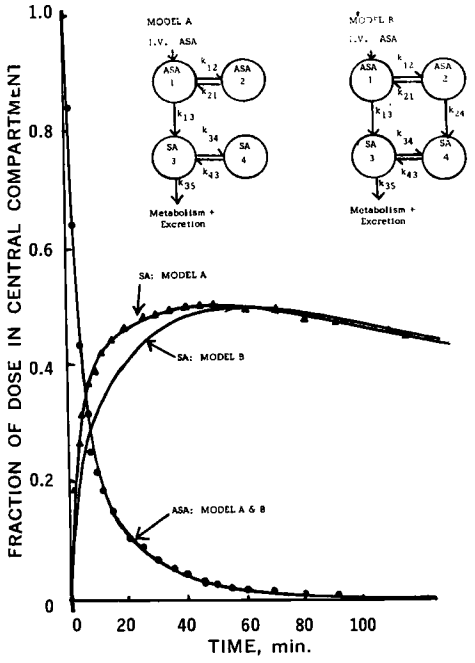

Fig. 6—Graph showing the observed and predicted values for the fraction of the dose as ASA and SA in the central compartment following i.v. administration of 650 mg. ASA. Model A assuming metabolism of ASA only in central compartment while model B assuming metabolism occurs in both compartments. Subject A. Key: ●, experimental ASA; ▲, experimental SA; ———, predicted analog computer curves. Model A, k_{12}, k_{21}, k_{13}, are 0.083, 0.104, 0.117 min.$^{-1}$, respectively. Model B, k_{12}, k_{21}, k_{13}, k_{24}, are 0.128, 0.094, 0.090, and 0.030 min.$^{-1}$, respectively, while k_{34}, k_{43}, and k_{35}, are 0.070, 0.105, and 0.0044 min.$^{-1}$ for both models.

therefore, concluded that the model proposed in Fig. 3 describes the observed kinetics of distribution, metabolism, and excretion of both ASA and SA.

The volume of distribution is another parameter which is used to characterize a drug. In terms of the two-compartmental model this is defined by the relationship (17).

$$V_{d_{ss}} = V_p \left(\frac{k_{12} + k_{21}}{k_{21}} \right) \qquad \text{(Eq. 1)}$$

where $V_{d_{ss}}$ is the volume of distribution at steady state of equilibrium, and is the only definition independent of k_{13}, the elimination rate constant. Assuming a single-compartmental model, the volume of distribution, V_d, is obtained by dividing the dose administered by the extrapolated intercept value for the concentration at zero time which, in terms of the two-compartmental model, is expressed by:

$$V_d = \frac{\text{dose}}{B} \qquad \text{(Eq. 2)}$$

The values of $V_{d_{ss}}$ and V_d for ASA and SA following their respective i.v. doses have been calculated and are listed in Table VI. Comparison of the respective values for ASA shows that the V_d may be twice as large as $V_{d_{ss}}$ due to significant metabolism of ASA occurring during the distribution phase of this drug, which is ignored in the single-compartment

TABLE VI—VALUES OF V_p[a], $V_{d_{ss}}$, AND V_d FOR ASA and SA

Subject	ASA V_p (L)	ASA $V_{d_{ss}}$ (L)	ASA V_d (L)	SA V_p (L)	SA $V_{d_{ss}}$ (L)	SA V_d (L)
A	5.6	10.1	21.0	5.3	8.7	8.8
B	6.9	12.0	17.6	5.9	9.7	9.7
C	6.5	10.6	16.3	5.2	9.1	9.1
D	6.5	10.4	19.7	5.7	10.2	10.4
E	7.6	13.3	30.2	—	—	—
Mean	6.6	11.3	21.0	5.5	9.4	9.5

[a] See text for definitions of terms.

model. Had the V_d term been used to compare these drugs, one could be led to the erroneous conclusion that the volume of distribution of ASA is almost twice that of SA. Contrary to this, the V_p and $V_{d_{ss}}$ for these two drugs are very similar with the ASA values being slightly larger than those of SA in each subject. This may be due to protein binding since SA has a much greater affinity for albumin than ASA (18) so that more SA would be held in the plasma and lower V_p and $V_{d_{ss}}$ would be expected. Other possible factors are differences in pKa and lipid solubility. Nevertheless, the difference between these drugs is not very great.

In contrast to ASA there is only a very small difference between the $V_{d_{ss}}$ and V_d for SA owing to negligible elimination occurring during the distribution phase of this drug. Therefore, with respect to the above calculations for SA either compartmental

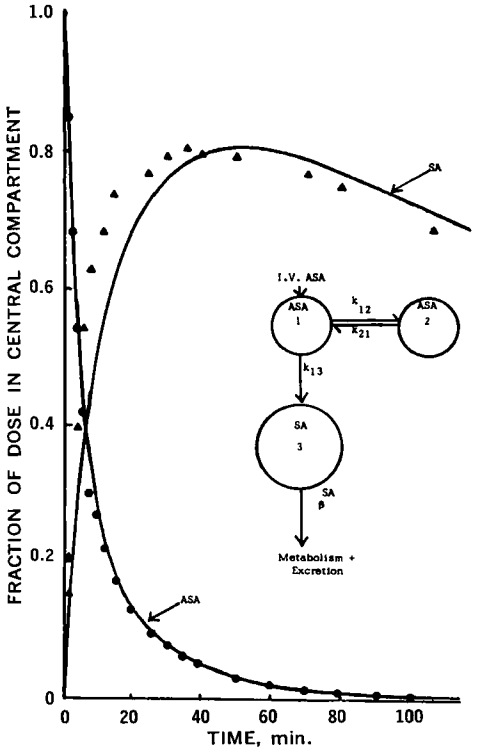

Fig. 7—Graph showing the observed and predicted values for the fraction of the dose and ASA and SA in the central compartment following i.v. administration of 650 mg. ASA and assuming single compartment for SA. Subject B. Key: ●, experimental ASA; ▲, experimental SA; ———, predicted analog computer curve.

model would be satisfactory. However, the two-compartmental model cannot be reduced to the single-compartmental model in all situations. If the latter model were substituted for SA in the schematic diagram describing the pharmacokinetics of ASA and SA (Fig. 3), the resultant predicted SA levels rise much more slowly than the observed values (Fig. 7). This results from the rapid metabolism of ASA to SA, such that SA builds up in the central compartment faster than it distributes. Hence, higher plasma levels are observed than one would expect using the single-compartment SA model which assumes spontaneous distribution of this drug. The V_p is a constant of the model, and although it is approximately 6 l. for either salicylate, it should not be equated to a physiological space such as the blood volume, even though their sizes are similar. Thus had whole blood been used instead of plasma, then as 70–80% of the salicylate is in the plasma (19), V_p values of approximately 10.1 would have been calculated.

Cummings (20, 21) observed that, following 0.65-g. plain aspirin tablets, peak plasma salicylate levels were at 1 hr. while excretion rate reached a maximum after 3 hr. To explain these results he suggested that distribution of SA was extremely slow in man, 3 or more hr. being required for equilibrium and that it was the tissue concentration, rather than the plasma, that controlled the excretion of this drug. Examination of the present data shows that distribution is very rapid and equilibrium (when tissue concentration is at a maximum) can be achieved within 20 min. following i.v. SA (Fig. 8). Probably, the explanation of Nelson and Levy (22) is correct in which they showed that the assay used by Cummings measures both SA and its glycine conjugate, salicyluric acid. Since plasma contains

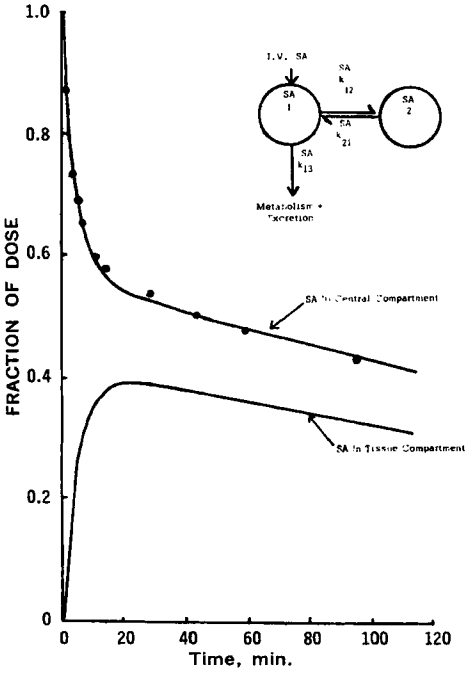

Fig. 8—Graph showing fraction of the dose in the central and tissue compartment following i.v. administration of SA. Subject B. Key: ●, experimental SA; ———, predicted analog computer curve.

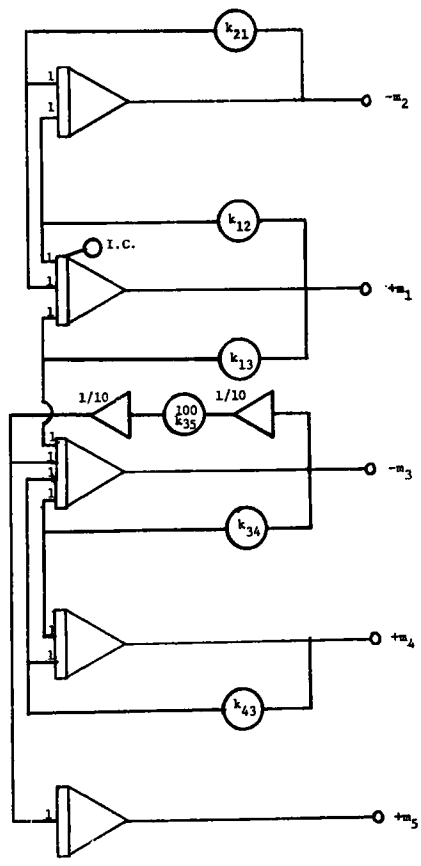

Fig. 9—Analog computer program.

predominately SA and urine mostly salicyluric acid (9) it follows from the precursor–product relationship that the maximum rate of excretion of total salicylate will be later than the peak plasma level.

An estimate of the contribution of whole blood to the *in vivo* hydrolysis of ASA in man can be made from the present data. Since the *in vitro* rate constant for the hydrolysis of ASA in human whole blood at 37° is 0.023 $min.^{-1}$ (23) and the blood volume is 5 l., the expected *in vivo* clearance (volume × rate constant) of ASA by blood is 115 ml./min. From the i.v. data the whole body clearance ($V_p \cdot k_{13}^{ASA}$) of ASA is 660 ml./min. (6,600 × 0.103). Consequently, whole blood only accounts for approximately 20% of total hydrolysis in man, and probably liver and perhaps kidney, are the major sites of ASA esterase activity. The implication that liver is the important metabolizing organ for ASA has been subsequently demonstrated (11). Obviously, as all the hepatic portal blood must pass through the liver this high hepatic clearance of ASA will significantly influence the amount of intact ASA which reaches the general circulation following oral administration of ASA.

Analysis of the present results has allowed a clearer understanding of the pharmacokinetics of ASA in man. Comparison shows that other than metabolic stability the pharmacokinetics of ASA and SA are very similar. However, even though the present model is useful, it must be emphasized that this model only describes the disposition kinetics

of SA at essentially one dose level, and consistency at one level is no test of a model. Since it is known that SA elimination kinetics do change with dose, more information is needed before a model can be proposed which will adequately describe the observed data at all dose levels of this drug.

APPENDIX

The mass equations describing the scheme in Fig. 3 are:

$$-\dot{m}_1 = k_{12} \cdot m_1 + k_{13} \cdot m_1 - k_{21} \cdot m_2 \quad \text{(Eq. 1a)}$$

$$\dot{m}_2 = k_{12} \cdot m_1 - k_{21} \cdot m_2 \quad \text{(Eq. 2a)}$$

$$\dot{m}_3 = k_{13} \cdot m_1 - k_{34} \cdot m_3 - k_{35} \cdot m_3 + k_{43} \cdot m_4 \quad \text{(Eq. 3a)}$$

$$-\dot{m}_4 = -k_{34} \cdot m_3 + k_{43} \cdot m_4 \quad \text{(Eq. 4a)}$$

$$-\dot{m}_5 = -k_{35} \cdot m_3 \quad \text{(Eq. 5a)}$$

$$\text{Dose} = m_1 + m_2 + m_3 + m_4 + m_5 \quad \text{(Eq. 6a)}$$

where m_x is the amount of durg in compartment x. The analog computer program for this scheme is shown in Fig. 9.

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Keyphrases

Pharmacokinetics—aspirin, salicylic acid
Aspirin, *N*-methylglucamine salt—IV administration
Salicylic acid, *N*-methylglucamine salt—IV administration
Two-compartmental open system—pharmacokinetic model
UV spectrophotometry—analysis
Fluorometry—analysis
GLC—analysis

Differential Ninhydrin Chromatographic Assay for the Gentamicin Complex

By GERALD H. WAGMAN, JANET V. BAILEY, and MORTON M. MILLER

A differential ninhydrin chromatographic assay has been devised to quantitatively determine the proportions of the three gentamicin components in gentamicin preparations. The technique consists of separation of the antibiotics on paper using the lower phase of a solvent system composed of chloroform, methanol, and 17 percent ammonium hydroxide (2:1:1 v/v). After development, strips containing the antibiotic are treated with ninhydrin reagent, developed, and color intensities read on an integrating scanner from which component proportions can be determined. Results are in excellent agreement with the microbiological method.

RECENTLY, Weinstein *et al.* (1) reported that preparations of the gentamicin complex consist of three antibiotic components which have been designated C_1, C_1a, and C_2 and that there are no significant differences in their biological properties. It became desirable therefore to devise methods for detection, isolation, and assay of these components. Thin-layer, paper, and column chromatographic separation techniques have been described by Wagman *et al.* (2) using a solvent system composed of chloroform–

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